OXFORD

Briefings in Functional Genomics, 15(3), 2016, 266–273

doi: 10.1093/bfgp/elv036
Advance Access Publication Date: 24 September 2015
Review paper

Roles of competing endogenous RNAs in gastric cancer

Ye Hu, Haiying Tian, Jie Xu and Jing-Yuan Fang

Corresponding authors: Jie Xu, Division of Gastroenterology and Hepatology in Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. Tel.: +86 (0) 21 53882322; E-mail: jiexu@yahoo.com and Jing-Yuan Fang, E-mail: jingyuanfang@sjtu.edu.cn

Abstract

Long noncoding RNA (lncRNA) is >200 nucleotides long and lacks coding ability. LncRNA was regarded as transcript noise, until emerging results showed its roles in development, homeostasis and carcinogenesis. LncRNAs containing microRNA (miRNA) response elements could compete with the miRNA target gene and regulate its expression through decreasing free functional miRNA. Such lncRNA is called competing endogenous RNA (ceRNA), and the 'lncRNA–miRNA' interaction appreciably enriches the world of RNA–RNA regulation. Gastric cancer involves dysregulation of both protein-coding genes and noncoding genes, and the ceRNA regulatory mechanism may participate in this pathogenic process. In this review, we discuss recent findings on the roles of ceRNAs in gastric carcinogenesis.

Key words: long noncoding RNA; competing endogenous RNA; microRNA binding sites; gastric cancer

Introduction

The human transcriptome is composed of only 2% of protein-coding RNA and 98% of noncoding RNA; however, the latter was considered as dark matter and did not draw the researchers' attention until recent years [1–5]. Long noncoding RNAs (lncRNAs) are functional RNAs deprived of coding potential; however, increasing evidence brings up the idea that lncRNAs could be an important class of permeative genes contributing to tumorigenesis [6]. Recent studies have reported diverse RNA species could form an intricate network, such as protein-coding messenger RNAs (mRNAs) and lncRNAs. These RNA transcripts could communicate with each other and pose reciprocal regulation by acting as competing endogenous RNAs (ceRNAs); in other words, these RNAs could compete to bind to common microRNAs (miRNAs), a cluster of small noncoding RNAs that are involved with posttranscriptional regulation of gene expression [7–12]. This mutual competition for miRNA leads to a decrease in free miRNA concentration, and thus an impairment of miRNA activity [13–15]. It is widely accepted that a single miRNA could regulate multiple target mRNAs through complementary binding to the 3′ untranslated region (3′-UTR) [16]. Conversely, a given mRNA could bear target sites for a considerable number of miRNAs, which could form a novel 'RNA–miRNA–RNA' interplay [17]. Understanding this complex RNA crosstalk in gene regulatory networks will provide significant insight into human development and diseases. Research on lncRNAs has witnessed a spectacular increase in these years. To date, long noncoding ceRNAs have been shown to function in several types of cancer including thyroid cancer, liver cancer, gallbladder cancer, etc [15, 18, 19]. Gastric cancer (GC) is one of the most prevalent cancer types worldwide. As the majority of patients have poor outcomes, it is crucial to understand the molecular mechanisms for GC and develop more effective therapeutic strategies. Here, we give an overview of the roles of lncRNAs as ceRNAs in GC.

Gastric cancer

GC is to date the fourth most common cancer and the second leading cause of cancer deaths worldwide [20–22]. However, the

Ye Hu received her doctoral degree in medicine from Renji Hospital affiliated to Shanghai Jiao Tong University School of Medicine. Her research is focused on the roles of lncRNAs in gastrointestinal tumors. She was involved in the discovery of GAPLINC, a ceRNA that regulates aggressiveness of gastric cancer.
Haiying Tian is a PhD candidate in Renji Hospital affiliated to Shanghai Jiao Tong University School of Medicine. She is interested in regulatory mechanisms of the TLR receptor signaling in gastrointestinal tumors.
Jie Xu is a Research Professor in Shanghai Institute of Digestive Disease, Shanghai Jiao Tong University School of Medicine. He is interested in the mechanisms of carcinogenesis in the GI system, and has characterized pathological functions of novel protein-coding genes and noncoding genes in GI tumors.
Jing-Yuan Fang is a Professor of Gastroenterology and Hepatology. He is the director of Division of Gastroenterology and Hepatology in Renji Hospital and the Shanghai Institute of Digestive Disease. His research is focused on the prevention, early diagnosis and intervention of gastric cancer and colorectal cancer.

patterns in GC vary substantially across geographical regions [22, 23], reflecting an uneven distribution of the key factors for GC incidence and mortality across different countries. The incidence and mortality of GC have decreased, mainly because of the reduction in the prevalence of *Helicobacter pylori* infection and smoking, and to the improvements in food preservation for the past years. However, GC remains a high burden for public health, especially in Asia, Latin America and Central and Eastern European areas [24, 25]. Our world witnesses great improvements in medical technologies and treatment strategies; however, major obstacles to the treatment of GC persist, including delayed diagnosis, recurrence and metastasis [26, 27]. Therefore, it is important to further clarify the molecular mechanisms of GC and to identify biomarkers for the diagnosis and prognosis of GC.

Carcinogenesis in the stomach is complex and includes multiple steps, involving numerous genetic and epigenetic alterations [28]. Studies over the past decades mostly focused on the protein-coding genes. The discovery of new functions in the noncoding RNAs (ncRNAs) is inducing a paradigm alteration in our understanding of gene regulation and its role in cancer development. LncRNAs are defined as ncRNAs with >200 nucleotides in length, and have emerged as a major class of regulatory factors that participate in a broad range of biological and pathological processes. LncRNAs have been subsequently reported to be involved in gene dysregulation in different types of cancers. The significance of lncRNAs in human GC was reported in 2012, when Feng and colleagues found the role of H19 in GC [29]. The H19 gene, which belongs to a highly conserved imprinted gene cluster [30, 31], was dramatically upregulated in GC cell lines and GC tissues. H19 was also found to promote GC cell proliferation [29]. Following this research, a series of studies focused on the aberrant expression of lncRNAs during GC have been reported. Accumulating results indicated that specific lncRNAs had potential pathological and clinical relevance in GC [32–39].

Competing endogenous RNA

The mysterious roles of lncRNAs used to be regarded as the dark matter of the genome which are gradually coming to light with our knowledge of the transcriptome space expanding. LncRNAs have been implicated in various ways of gene regulation, such as chromatin dosage compensation, epigenetic regulation, mRNA splicing and translation [40–42]; thus, dysregulation of these key regulators would inevitably lead to diseases. It has become increasingly evident that a wide range of RNA transcripts bearing a number of miRNA-binding sites, contributing to the hypothesis that all RNA transcripts that contain miRNA-binding sites could connect with and influence each other by competing specifically for common miRNAs, can thus act as ceRNAs [17, 43, 44] (schematic representation in Figure 1). The previous mode, 'miRNAs–RNAs' regulation, now has been evolved to 'RNAs–miRNAs–RNAs' interaction [17].

The ceRNA network has three main characteristics. The first one is that the relative concentration of the ceRNAs and corresponding miRNAs could appreciably impact the effectiveness of the interaction. Altered expression of the ceRNAs in different conditions should reach a certain level, to cause or relieve the miRNA suppression on ceRNAs. Second, the range of miRNAs that a ceRNA may interact largely depends on the subcellular localization and tissue/cell specificity of ceRNA [45]. As the expression of miRNA is dependent on time, cell/tissue type, developmental stage and pathological context, the availability of ceRNA under these conditions would also affect its overall influence [46]. Third, the miRNA response elements (MREs) on ceRNAs are not equal. For example, two different MREs are predicted to bind the same miRNA; the partial difference in nucleotide composition of MREs could lead to distinct affinity of each MRE to bind a miRNA and is critical for overall ceRNA function [17].

A multitude of lncRNAs have displayed cell type-, tissue type-, developmental stage- and disease-specific expression

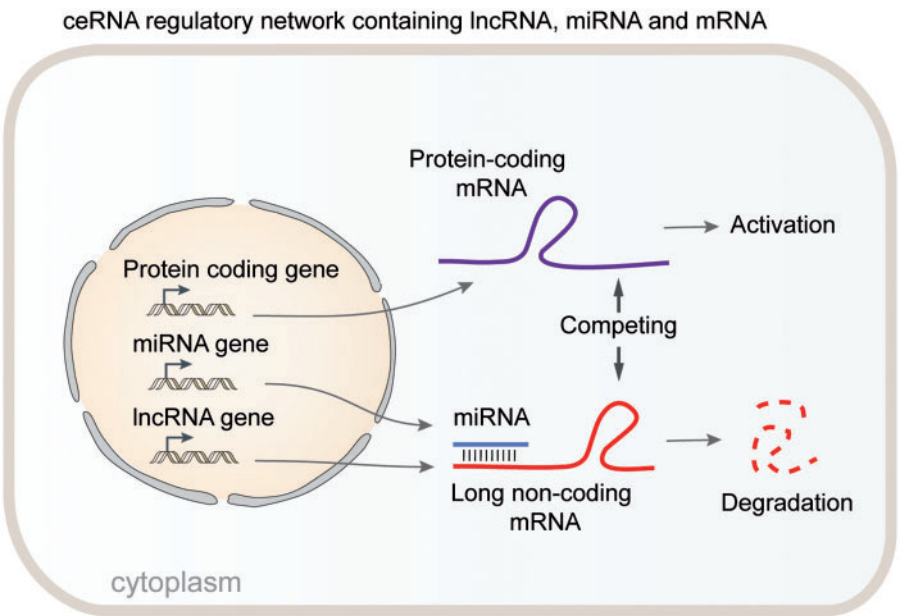

Figure 1. Schematic representation for the effects of long noncoding ceRNA on miRNA and protein-coding RNA.
Both the long noncoding ceRNA and protein-coding mRNA contain binding sites (MREs) for the miRNA, and the upregulation of long noncoding ceRNA competes with protein-coding RNA to bind miRNA. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

modes [15, 18, 19, 47], suggesting that individual lncRNA could be a potent natural miRNA decoy in certain conditions. Two such examples of long noncoding ceRNAs that have emerged, highlighting the significance of these miRNA–lncRNA competitive crosstalk, are CCAT1 and PTCSC3. The lncRNA CCAT1 was first reported as a significantly upregulated transcript in colorectal cancer (CRC), with 2628 bp length that maps to chromosome 8q24.21 [48]. Moreover, abnormal upregulation of CCAT1 promotes cell proliferation and migration in GC cells [32]. More recently, CCAT1 was demonstrated to be highly expressed in gallbladder carcinoma tissues. CCAT1 promoted the miRNA-218-5p target gene Bmi1 by competitively binding to miRNA-218-5p, and CCAT1 could significantly stimulate the proliferation and invasiveness of GBC cells. In contrast to CCAT1 that is upregulated in cancerous tissues, PTCSC3 was found to be dramatically downregulated in thyroid cancers [19]. PTCSC3 expression is highly thyroid specific. Overexpression of PTCSC3 in thyroid cancer cells inhibits cell growth and stimulates cell cycle arrest and apoptosis, suggesting that PTCSC3 plays a crucial role in thyroid carcinogenesis. Further investigation revealed PTCSC3 could bind miR-574-5p, which was reported to accelerate proliferation of several human cancer cells and exert influence on CRC tumorigenesis, and it could serve as a biomarker for chemoresistance and poor survival in patients with small-cell lung cancer [49–51]. These two studies indicate that the disease-specific alteration of specific lncRNAs and associated ceRNA crosstalk may effect on the carcinogenic process.

ceRNA in GC

Theoretically, any RNA transcript that bears MREs is capable of sequestering miRNAs and constructing intricate ceRNA–miRNA networks that when perturbed may contribute to cancer [8, 52]. Growing evidence suggests that lncRNAs could also have roles as ceRNAs, connecting miRNAs and the posttranscriptional crosstalk in gastric pathogenesis. Explicating this novel RNA interplay will provide new insight into the underlying mechanisms of tumorigenesis and pave the way for developing new therapeutic methods against GC.

Long noncoding ceRNA—HOTAIR

HOTAIR is as a spliced and polyadenylated lncRNA containing 2158 nucleotides [53]. HOTAIR is transcribed from the antisense strand of HoxC gene, and it is a trans-acting lncRNA with different target loci [53, 54]. HOTAIR initially became well known for its oncogenic functions in primary and metastatic breast cancer [55]. Furthermore, HOTAIR expression level positively correlates with malignant behavior and poor prognosis in diverse cancers such as hepatocellular carcinoma and pancreatic cancer [56–61]. Recent studies showed that HOTAIR was upregulated in GC [62, 63]. Additionally, its experimental upregulation promoted GC cells proliferation, migration and invasion, whereas its inhibition dramatically turned back the promotion. Hyperactivity of HOTAIR was correlated with bigger tumor dimensions, higher disease stage, prolonged metastasis status and shorter survival of GC patients.

Mechanistic analysis reveals that nuclear HOTAIR can target polycomb repressive complex 2, altering H3K27 methylation and gene expression patterns across the genome [55, 56]. More interestingly, HOTAIR could also function as a ceRNA to compete for miR-331-3p in gastric pathogenesis [64] (Figure 2). Bioinformatics analysis of miRNA recognition sequences on HOTAIR revealed the presence of 11 tumor-suppressive miRNAs-binding sites. MiRNAs are known to be present in the cytoplasm in the form of miRNA ribonucleoprotein complexes that also contain Ago2, the core component of the RNA-induced silencing complex [65, 66]. HOTAIR: miR-331-3P coimmunoprecipitation with anti-Ago2 demonstrated a physical interaction in GC cells, providing further support for HOTAIR's miRNA-sequestering activity.

In carcinomas, human epithelial growth factor receptor 2 (HER2) encodes a 185 kDa transmembrane protein that triggers

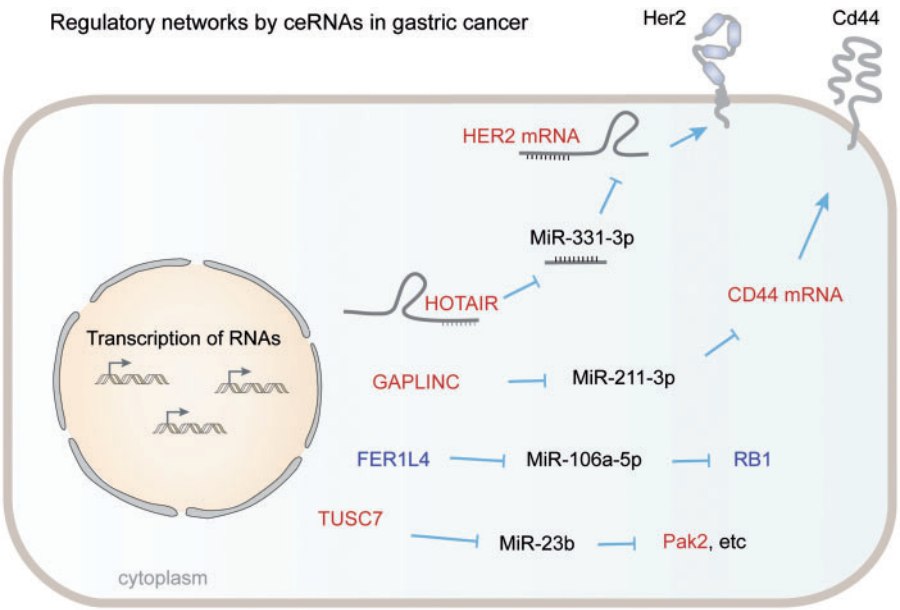

Figure 2. GC-related signaling pathways formed by long noncoding ceRNAs, miRNAs and protein-coding mRNAs. The pro-oncogenic long noncoding ceRNAs HOTAIR and GAPLINC, respectively, compete with HER2 (ERBB2) and CD44 to bind miR-331-3p and miR-211-3p. Upregulation of long noncoding ceRNAs leads to activation of Her2 and Cd44, which promote gastric carcinogenesis. The tumor-suppressive long noncoding ceRNA FER1L4 upregulates RB1 tumor suppressor by binding to miR-106a-5p. Reciprocal repression has been found between TUSC7 and miR-23b in GC. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

multiple signaling pathways involved in cell proliferation, apoptosis, motility and angiogenesis [67–70]. HER2 was predicted to be one of miR-211-3p target genes, with luciferase assay to prove this interaction. Bivariate correlation analysis showed that expression of HER2 was significantly correlated with HOTAIR transcript level in GC tissues compared with normal counterparts. This indicates that HOTAIR acts as ceRNA by binding miR-311-3p, thus abolishing the miRNA-induced suppressing activity on the HER2 3′-UTR.

Thus, the identification of long noncoding ceRNAs will undoubtedly enhance our knowledge of how lncRNAs function, allowing us to better understand the pathogenesis and development of GC and finally facilitate the development of lncRNA-based diagnostics and therapeutics against this deadly disease. It is reasonable for us to anticipate that there may be many other lncRNAs that function as ceRNAs to regulate expression of key genes in GC.

Long noncoding ceRNA—GAPLINC

Our previous study found that GAPLINC, a long intergenic noncoding RNA (lincRNA) sitting on the shorter arm of chromosome 18 (924 bp long), is highly expressed in GC tissues with cancer-predictive value. Experimental validation based on patients with different clinicopathologic features suggested that GAPLINC high expression was a strong indicator of poor prognosis, and it was correlated with larger average tumor size, more frequent of severe lymph node invasion and shorter patient survival [71].

GAPLINC repression could significantly inhibit the proliferation and invasion of GC cell lines MGC803 and SGC7901, whereas knocking down of GAPLINC facilitates apoptosis and cycle arrest in both cell lines. GAPLINC might be a facilitating factor in the gastric carcinogenesis and development, with gene expression profiling in GC cells revealing alterations in cell migration pathways and with CD44 expression as the most highly correlated after downregulation of GAPLINC. CD44 is a well-characterized factor that contributes to a broad range of tumor occurrence and progression, including GC [72–76]. Overexpression of GAPLINC increased CD44 mRNA level in GC cells, and the effects of GAPLINC on cell migration and proliferation were suppressed by interfering CD44 mRNA [71]. Through the high-throughput ArrayStar assay, we are the first group to uncover that GAPLINC is not only a strong indicator, but also a main factor contributing to GC occurrence and progression. Therefore, probing into the mechanism of this novel lincRNA regulating its downstream gene CD44 might provide us with more information of lncRNA taking part in GC.

Interestingly, a miRNA, namely miR211-3p, was predicted to target both CD44 and GAPLINC, with remarkable binding energy estimated by the widely used RNAup algorithm [77] (Figure 2). Further mechanistic investigations validated the ceRNA hypothesis that GAPLINC regulates CD44 as a molecular sponge for miR211-3p. The examples of GAPLINC and HOTAIR representing as ceRNAs give us a hint that a wide variety of lncRNA might exert its regulation through acting as ceRNA in GC.

Long noncoding ceRNA—tumor suppressor candidate 7

Another long noncoding ceRNA example is tumor suppressor candidate 7 (TUSC7), which was first discovered by Qi and colleagues in 2015 [78] (Figure 2). The author first performed an lncRNA microarray in eight GC and paired paracancerous tissues, and used gene coexpression network to identify potential lncRNA. TUSC7 was found out to be linked to a number of lncRNAs and mRNAs; thus, it was chosen to study on. Sequential analysis demonstrated that low expression of TUSC7 was associated with more aggressive behavior and poorer prognosis in GC patients, such as a worse pathologic grade, a deeper and nervous invasion. The Kaplan–Meier method with the log-rank test showed that decreased TUSC7 expression was an independent risk factor of disease-free survival and disease-specific survival in GC patients. Additionally, TUSC7 was underexpressed in GC samples compared with corresponding normal gastric tissues, indicating a protective role of TUSC7. Further functional study revealed that TUSC7 suppressed tumor cell growth both *in vitro* and *in vivo*. The p53 tumor suppressor was predicted and experimentally validated to be the upstream transcription factor via interaction with the putative p53-response element in the upstream region of TUSC7. Ultimately, through a series of processes, including miRNA prediction, RNA expression detection and RNA immunoprecipitation, miR-26b was indicated to bind to TUSC7. It should be noted that miR-23b could not only significantly promote cell growth and but rescue the effects of TUSC7 overexpression. It has been demonstrated that TUSC7 and miR-23b were expressed in negative correlation in clinical specimens, and could pose reciprocal repression. Altogether, TUSC7 is a p53-regulated tumor suppressor that acts partially by decreasing miR-23b, and TUSC7 may be a key regulatory factor in GC. However, the other side of the ceRNA-target genes of miR-23b was not proposed in this study.

Identification of long noncoding ceRNA–miRNA interaction

Another study aimed to develop a new approach to construct ceRNA network and to make it easier to search for cancer-associated long noncoding ceRNAs [79] (flowchart shown in Figure 3). The study focused on GC-associated lncRNAs; thus, 17 lncRNAs differentially expressed between GC tissues and paracancerous tissues were selected on the lncRNAs expression profiles combined with Encyclopedia of DNA Elements [80]. Applying the previously reported GC-associated miRNAs [81], the author tried to discover these miRNA-associated long noncoding ceRNAs. Because miRNAs are interacting with lncRNAs through their MREs within ceRNA network, it is a matter of great urgency to search for the potential MREs in lncRNAs. The ceRNA hypothesis relies on knowledge of the precise number and location of MREs. This study applied miRcode [82] to identify miRNA targets, and it showed that 13 miRNAs may interact with 9 of 17 lncRNAs; thus, a ceRNA network *in silico* was constructed. To establish lncRNA–miRNA–mRNA network (ceRNA network), the next step was to search for miRNAs' mRNA targets. Based on those miRNAs that might interact with lncRNAs, the researchers explored miRNAs' mRNA targets with experimental support tool using TarBase [83]. The results showed that nine miRNAs and eight lncRNAs may be included in the RNA–RNA regulated complex. To confirm this network, a regression analysis based on the gene expression data of several other types of cancer, including head and neck squamous cell carcinoma, prostate cancer, papillary thyroid carcinoma, etc. Finally, several experimental data were also included to prove the ceRNA network. This work revealed that FER1L4 ceRNA interacted with RB1 mediated by miR-106a-5p, and pointed out that H19 might interact with MYCN ceRNA mediated by miR-19a-3p,

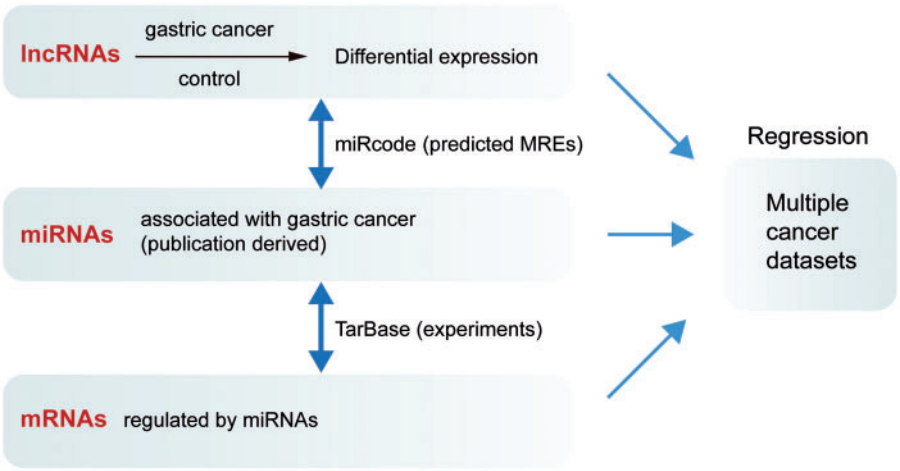

Figure 3. Flowchart of the method by for identification of ceRNA networks in GC. The differentially expressed lncRNAs were determined by comparing lncRNA expression between cancer and control tissue samples, and the miRNAs associated with GC were according to published information. The potential interactions between lncRNA and miRNAs were predicted by miRcode tool that is based on MREs on lncRNAs. The TarBase (based on experimental results) was used to identify mRNAs regulated by miRNAs. Finally, the relationships between lncRNA, miRNA and mRNA were analyzed by multiple cancer data sets. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

Table 1. Tools and resources for ceRNAs in cancer

Tool name	Version	Platform	Developer	Applicable areas/functions	Reference (PMID)
Cupid	N/A	MATLAB	The University of Texas; Columbia University	Simultaneous prediction of miRNA-target interactions and ceRNA interactions	25378249
Hermes	2.0	MATLAB	Columbia University	Predicts ceRNA interactions from expression profiles of candidate RNAs and their common miRNA regulators using conditional mutual information	22000015
starBase	2.0	Web tool	Sun Yat-sen University, China	Predicts miRNA–ceRNA, miRNA–ncRNA and protein–RNA interaction networks from large-scale CLIP-Seq data	24297251
Cefinder	N/A	Web tool	University of Minnesota	Identifies ceRNA for a given mRNA target based on the homology of miRNA-binding sites present in the 3′-UTR sequence	23055620
InCeDB	N/A	Database	Indian Association for the Cultivation of Science, India	Database of human lncRNA acting as ceRNA	24926662
Linc2GO	N/A	Database	Tsinghua University, China	A human lincRNA function annotation resource based on ceRNA hypothesis	23793747

and LINC00152 ceRNA might interact with THBS1 mediated by miR-18a-5p. Although several ceRNA interactions were predicted without experimental validation, this new approach of predicting cancer associated-ceRNA network may provide useful information for discovering interactive RNAs that may functionally compete with each other.

Several dedicated tools have been developed to facilitate the identification of ceRNA networks, including Cupid [84], Hermes [85], starBase [86], cefinder [87], inCeDB [88] and Linc2GO [89]. The features of these tools and resources have been summarized in Table 1. With the advances of bioinformatic tools, the identification of cancer-related ceRNA networks would be more efficient and accurate.

Conclusion

The ceRNA hypothesis was first brought up in the year 2011 and was rapidly considered as a hallmark in the general understanding the world of RNA–RNA regulation. CeRNA-based gene regulation is an arising area of investigation that would remarkably rise our comprehension of cancer molecular mechanism.

The molecular mechanisms of GC have been better understood in the past two decades [90]; however, a large number of GC are diagnosed in advanced stages, leading to the loss of the opportunity for radical surgery and poor prognosis and unsatisfactory survival [91]. Generally, multiple genes and signaling pathways are disrupted in GC [92]. Upregulation of ceRNAs raises the abundance of specific MREs and shifts the miRNA pool distribution, as a result, leading to the increased expression of target mRNA. Given the crucial roles of transcriptome alterations in carcinogenesis and the pervasiveness of ceRNA interplay, it is sensible for us to anticipate an increasing number of ceRNA networks being identified in GC. The effectiveness of ceRNA crosstalk may be influenced by several factors such as the relative level of miRNA abundance and ceRNA abundance, and subcellular localization of the individual molecule.

HOTAIR is a widely known lncRNA to play vital role in the etiology of various types of cancer, and recent researches revealed this well-known factor could act as ceRNA to exert its

influence on target genes. GAPLINC and TUSC7 are newly reported lncRNAs, and could also take on the ceRNA role in GC occurrence and development. Since the ceRNA hypothesis was proposed, several tools have been developed to help the discovery of ceRNA network in cancer.

As a summary, the novel connection between alternative gene expression and long noncoding ceRNA network further highlights the advantages of understanding tumorigenesis from a systematic perspective. The study on the roles of long noncoding ceRNA in GC is still in its early stage, and future explorations may provide more mechanistic insights and potential biomarkers for GC.

Key Points

- LncRNAs containing miRNA-binding sites could act as ceRNAs and play a vital role in gastric carcinogenesis and progression.
- CeRNAs could communicate with each other and pose reciprocal regulation through competing to bind to common miRNAs, which forms a novel 'RNA–miRNA–RNA' interplay.
- So far, only a few of lncRNAs, such as HOTAIR, GAPLINC and TUSC7 are reported as ceRNAs in GC, but more ceRNAs are waiting to be explored.
- A number of bioinformatics tools are developed to help us discover the ceRNA interaction, for example, miRcode could be used to search for miRNA binding to lncRNA, and TarBase could be used to find miRNA target genes.

Funding

This project was supported by grants from National Natural Science Foundation of China (30971330, 31371420, 81320108024, 81000861, 81322036, and 81272383); Shanghai 'Oriental Scholars' project (2013XJ).

References

1. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* 2014;**157**:77–94.
2. Morris KV, Mattick JS. The rise of regulatory RNA. *Nat Rev Genet* 2014;**15**:423–37.
3. Sabin LR, Delás MJ, Hannon GJ. Dogma derailed: the many influences of RNA on the genome. *Mol Cell* 2013;**49**:783–94.
4. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet* 2011;**12**:99–110.
5. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem* 2012;**81**:145–66.
6. Bánfai B, Jia H, Khatun J, *et al.* Long noncoding RNAs are rarely translated in two human cell lines. *Genome Res* 2012;**22**:1646–57.
7. Baltimore D. Our genome unveiled. *Nature* 2001;**409**:814–16.
8. Poliseno L, Salmena L, Zhang J, *et al.* A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* 2010;**465**:1033–8.
9. Chi SW, Zang JB, Mele A, *et al.* Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature* 2009;**460**:479–86.
10. Licatalosi DD, Mele A, Fak JJ, *et al.* HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 2008;**456**:464–9.
11. Friedman RC, Farh KK, Burge CB, *et al.* Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009;**19**:92–105.
12. Thomas M, Lieberman J, Lal A. Desperately seeking microRNA targets. *Nat Struct Mol Biol* 2010;**17**:1169–74.
13. Cazalla D, Yario T, Steitz J. Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. *Science* 2010;**328**:1563–6.
14. Lee DY, Jeyapalan Z, Fang L, *et al.* Expression of versican 3′-untranslated region modulates endogenous microRNA functions. *PloS One* 2010;**5**:e13599.
15. Wang J, Liu X, Wu H, *et al.* CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res* 2010;**38**:5366–83.
16. Baek D, Villén J, Shin C, *et al.* The impact of microRNAs on protein output. *Nature* 2008;**455**:64–71.
17. Salmena L, Poliseno L, Tay Y, *et al.* A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011;**146**:353–8.
18. Ma MZ, Chu BF, Zhang Y, *et al.* Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. *Cell Death Dis* 2015;**6**:e1583.
19. Fan M, Li X, Jiang W, *et al.* A long non-coding RNA, PTCSC3, as a tumor suppressor and a target of miRNAs in thyroid cancer cells. *Exp Ther Med* 2013;**5**:1143–6.
20. Bertuccio P, Chatenoud L, Levi F, *et al.* Recent patterns in gastric cancer: a global overview. *Int J Cancer* 2009;**125**:666–73.
21. Bosetti C, Bertuccio P, Malvezzi M, *et al.* Cancer mortality in Europe, 2005–2009, and an overview of trends since 1980. *Ann Oncol* 2013;**24**:2657–71.
22. Peleteiro B, Severo M, La Vecchia C, *et al.* Model-based patterns in stomach cancer mortality worldwide. *Eur J Cancer Prev* 2014;**23**:524–31.
23. Bastos J, Peleteiro B, Barros R, *et al.* Sociodemographic determinants of prevalence and incidence of *Helicobacter pyloriinfection* in Portuguese adults. *Helicobacter* 2013;**18**:413–22.
24. Peleteiro B, La Vecchia C, Lunet N. The role of *Helicobacter pylori* infection in the web of gastric cancer causation. *Eur J Cancer Prev* 2012;**21**:118–25.
25. Siegel R, Ma J, Zou Z, *et al.* Cancer statistics. *CA Cancer J Clin* 2014;**64**:9–29.
26. Shen L, Shan YS, Hu HM, *et al.* Management of gastric cancer in Asia: resource-stratified guidelines. *Lancet Oncol* 2013;**14**:e535–47.
27. Thiel A, Ristimaki A. Gastric cancer: basic aspects. *Helicobacter* 2012;**17**:26–9.
28. Stock M, Otto F. Gene deregulation in gastric cancer. *Gene* 2005;**360**:1–19.
29. Yang F, Bi J, Xue X, *et al.* Up-regulated long non-coding rna h19 contributes to proliferation of gastric cancer cells. *FEBS J* 2012;**279**:3159–65.
30. Bartolomei MS, Zemel S, Tilghman SM. Parental imprinting of the mouse h19 gene. *Nature* 1991;**351**:153–5.
31. Gabory A, Jammes H, Dandolo L. The h19 locus: role of an imprinted non-coding RNA in growth and development. *BioEssays News Rev Mol Cell Dev Biol* 2010;**32**:473–80.
32. Yang F, Xue X, Bi J, *et al.* Long noncoding RNA CCAT1, which could be activated by c-Myc, promotes the progression of gastric carcinoma. *J Cancer Res Clin Oncol* 2013;**139**:437–45.
33. Yang F, Xue X, Zheng L, *et al.* Long non-coding RNA GHET1 promotes gastric carcinoma cell proliferation by increasing c-Myc mRNA stability. *FEBS J* 2014;**281**:802–13.
34. Xu ZY, Yu QM, Du YA, *et al.* Knockdown of long non-coding RNA HOTAIR suppresses tumor invasion and reverses

epithelial-mesenchymal transition in gastric cancer. *Int J Biol Sci* 2013;**9**:587–97.
35. Zhang EB, Kong R, Yin DD, *et al*. Long noncoding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor growth by epigenetically silencing of miR-99a/miR-449a. *Oncotarget* 2014;**5**:2276–92.
36. Zhao Y, Guo Q, Chen J, *et al*. Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: a clinical and in vitro investigation. *Oncol Rep* 2014;**31**:358–64.
37. Sun M, Xia R, Jin F, *et al*. Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. *Tumour Biol* 2014;**35**:1065–73.
38. Sun M, Jin FY, Xia R, *et al*. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. *BMC Cancer* 2014;**14**:319.
39. Wang J, Su L, Chen X, *et al*. MALAT1 promotes cell proliferation in gastric cancer by recruiting SF2/ASF. *Biomed Pharmacother* 2014;**68**:557–64.
40. Harries LW. Long non-coding RNAs and human disease. *Biochem Soc Trans* 2012;**40**:902–6.
41. Clark MB, Mattick JS. Long noncoding RNAs in cell biology. *Semin Cell Dev Biol* 2011;**22**:366–76.
42. Moran VA, Perera RJ, Khalil AM. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. *Nucleic Acids Res* 2012;**40**:6391–400.
43. Ebert MS, Sharp PA. Emerging roles for natural microRNA sponges. *Curr Biol* 2010;**20**:R858–61.
44. Seitz H. Redefining microRNA targets. *Curr Biol* 2009;**19**:870–3.
45. Wee LM, Flores-Jasso CF, Salomon WE, *et al*. Argonaute divides its RNA guide into domains with distinct functions and RNA-binding properties. *Cell* 2012;**151**:1055–67.
46. Venables JP, Klinck R, Koh C, *et al*. Cancer-associated regulation of alternative splicing. *Nat Struct Mol Biol* 2009;**16**:670–6.
47. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature* 2012;**482**:339–46.
48. Nissan A, Stojadinovic A, Mitrani-Rosenbaum S, *et al*. Colon cancer associated transcript-1: a novel RNA expressed in malignant and pre-malignant human tissues. *Int J Cancer* 2012;**130**:1598–606.
49. Meyers-Needham M, Ponnusamy S, Gencer S, *et al*. Concerted functions of HDAC1 and microRNA-574-5p repress alternatively spliced ceramide synthase 1 expression in human cancer cells. *EMBO Mol Med* 2012;**4**:78–92.
50. Ranade AR, Cherba D, Sridhar S, *et al*. MicroRNA 92a-2*: a biomarker predictive for chemoresistance and prognostic for survival in patients with small cell lung cancer. *J Thorac Oncol* 2010;**5**:1273–8.
51. Ji S, Ye G, Zhang J, *et al*. miR-574-5p negatively regulates Qki6/7 to impact β-catenin/Wnt signalling and the development of colorectal cancer. *Gut* 2013;**62**:716–26.
52. Tay Y, Kats L, Salmena L, *et al*. Coding- independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell* 2011;**147**:344–57.
53. Rinn JL, Kertesz M, Wang JK, *et al*. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 2007;**129**:1311–23.
54. Hajjari M, Khoshnevisan A, Shin YK. Molecular function and regulation of long non-coding RNAs: paradigms with potential roles in cancer. *Tumour Biol* 2014;**35**:10645–63.
55. Gupta RA, Shah N, Wang KC, *et al*. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010;**464**:1071–6.
56. Kogo R, Shimamura T, Mimori K, *et al*. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. *Cancer Res* 2011;**71**:6320–6.
57. Geng YJ, Xie SL, Li Q, *et al*. Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression. *J Int Med Res* 2011;**39**:2119–28.
58. Kim K, Jutooru I, Chadalapaka G, *et al*. HOTAIR is a negative prognostic factor and exhibits pro- oncogenic activity in pancreatic cancer. *Oncogene* 2013;**32**:1616–25.
59. Niinuma T, Suzuki H, Nojima M, *et al*. Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors. *Cancer Res* 2012;**72**:1126–36.
60. Huang L, Liao LM, Liu AW, *et al*. Overexpression of long noncoding RNA HOTAIR predicts a poor prognosis in patients with cervical cancer. *Arch Gynecol Obstet* 2014;**290**:717–23.
61. Nie Y, Liu X, Qu S, *et al*. Long non-coding RNA HOTAIR is an independent prognostic marker for nasopharyngeal carcinoma progression and survival. *Cancer Sci* 2013;**104**:458–64.
62. Endo H, Shiroki T, Nakagawa T, *et al*. Enhanced expression of long non-coding RNA HOTAIR is associated with the development of gastric cancer. *PLoS One* 2013;**8**:e77070.
63. Hajjari M, Behmanesh M, Sadeghizadeh M, *et al*. Up-regulation of HOTAIR long non-coding RNA in human gastric adenocarcinoma tissues. *Med Oncol* 2013;**30**:670.
64. Liu XH, Sun M, Nie FQ, *et al*. LncRNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. *Mol Cancer* 2014;**13**:92.
65. Izaurralde E. Elucidating the temporal order of silencing. *EMBO Rep* 2012;**13**:662–3.
66. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008;**9**:102–14.
67. Lee HE, Park KU, Yoo SB, *et al*. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. *Eur J Cancer* 2013;**49**:1448–57.
68. Huang TH, Morrison SL. A trimeric anti-HER2/neu ScFv and tumor necrosis factor-alpha fusion protein induces HER2/neu signaling and facilitates repair of injured epithelia. *J Pharmacol Exp Ther* 2006;**316**:983–91.
69. Lemoine NR, Jain S, Silvestre F, *et al*. Amplification and overexpression of the EGF receptor and c-erbB-2 proto-oncogenes in human stomach cancer. *Br J Cancer* 1991;**64**:79–83.
70. Faltus T, Yuan J, Zimmer B, *et al*. Silencing of the HER2/neu gene by siRNA inhibits proliferation and induces apoptosis in HER2/neu-overexpressing breast cancer cells. *Neoplasia* 2004;**6**:786–95.
71. Hu Y, Wang J, Qian J, *et al*. Long noncoding RNA GAPLINC regulates CD44-dependent cell invasiveness and associates with poor prognosis of gastric cancer. *Cancer Res* 2014;**74**:6890–902.
72. Loh TJ, Moon H, Cho S, *et al*. CD44 alternative splicing and hnRNP A1 expression are associated with the metastasis of breast cancer. *Oncol Rep* 2015;**34**:1231–8.
73. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. *Adv Cancer Res* 1997;**71**:241–319.
74. Paulis YW, Huijbers EJ, van der Schaft DW, *et al*. CD44 enhances tumor aggressiveness by promoting tumor cell plasticity. *Oncotarget* 2015;**6**:19634–46.
75. Wu Y, Li Z, Zhang C, *et al*. CD44 family proteins in gastric cancer: a meta-analysis and narrative review. *Int J Clin Exp Med* 2015;**8**:3595–606.

76. Bertaux-Skeirik N, Feng R, Schumacher MA, *et al*. CD44 plays a functional role in *Helicobacter pylori*-induced epithelial cell proliferation. *PLoS Pathog* 2015;**11**:e1004663.
77. Muckstein U, Tafer H, Hackermuller J, *et al*. Thermo dynamics of RNA–RNA binding. *Bioinformatics* 2006;**22**:1177–82.
78. Qi P, Xu MD, Shen XH, *et al*. Reciprocal repression between TUSC7 and miR-23b in gastric cancer. *Int J Cancer* 2015;**137**:1269–78.
79. Xia T, Liao Q, Jiang X, *et al*. Long noncoding RNA associated-competing endogenous RNAs in gastric cancer. *Sci Rep* 2014;**4**:6088.
80. Harrow J, Frankish A, Gonzalez JM, *et al*. GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res* 2012;**22**:1760–74.
81. Guo J, Miao Y, Xiao B, *et al*. Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. *J Gastroenterol Hepatol* 2009;**24**:652–7.
82. Jeggari A, Marks DS, Larsson E. miRcode: a map of putative microRNA target sites in the long non-coding transcriptome. *Bioinformatics* 2012;**28**:2062–3.
83. Vergoulis T, Vlachos IS, Alexiou P, *et al*. TarBase 6.0: capturing the exponential growth of miRNA targets with experimental support. Nucleic Acids Res. 2012;**40**:D222–9.
84. Chiu HS, Llobet-Navas D, Yang X, *et al*. Cupid: simultaneous reconstruction of microRNA-target and ceRNA networks. *Genome Res* 2015;**25**:257–67.
85. Sumazin P, Yang X, Chiu HS, *et al*. An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. *Cell* 2011;**147**:370–81.
86. Li JH, Liu S, Zhou H, *et al*. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 2014;**42**:D92–7.
87. Sarver AL, Subramanian S. Competing endogenous RNA database. *Bioinformation* 2012;**8**:731–3.
88. Das S, Ghosal S, Sen R, Chakrabarti J. lnCeDB: database of human long noncoding RNA acting as competing endogenous RNA. *PLoS One* 2014;**9**:e98965.
89. Liu K, Yan Z, Li Y, *et al*. Linc2GO: a human lincRNA function annotation resource based on ceRNA hypothesis. *Bioinformatics* 2013;**29**:2221–2.
90. Qian J, Kong X, Deng N, *et al*. OCT1 is a determinant of synbindin-related ERK signalling with independent prognostic significance in gastric cancer. *Gut* 2015;**64**:37–48.
91. Kong X, Qian J, Chen LS, *et al*. Synbindin in extracellular signal-regulated protein kinase spatial regulation and gastric cancer aggressiveness. *J Natl Cancer Inst* 2013;**105**:1738–49.
92. Liang L, Fang JY, Xu J. Gastric cancer and gene copy number variation: emerging cancer drivers for targeted therapy. *Oncogene* 2016;**35**:1475–82.